

1. A nucleic acid molecule comprising:

(A) a first nucleotide sequence encoding an AAV Rep protein of a first serotype;

(B) a second nucleotide sequence encoding an AAV Cap protein of a second serotype;

the second serotype being different from the first serotype; and

5 (C) a third nucleotide sequence encoding a transcription product having at least one Adenoviral helper function.

2. The nucleic acid molecule of claim 1, wherein the nucleic acid molecule is comprised within a vector.

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3. The nucleic acid molecule of claim 1, wherein the AAV Rep protein is an AAV serotype 2 protein.

4. The nucleic acid molecule of claim 1, wherein the AAV Rep protein is Rep52.

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5. The nucleic acid molecule of claim 1, wherein the AAV Rep protein is Rep78.

6. The nucleic acid molecule of claim 4, wherein the first nucleotide sequence additionally encodes a Rep78 protein.

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7. The nucleic acid molecule of claim 1, wherein the AAV Cap protein is an AAV serotype 1 Cap protein.

8. The nucleic acid molecule of claim 1, wherein the AAV Cap protein is an AAV serotype 5 Cap protein.
9. The nucleic acid molecule of claim 1, wherein the second nucleotide sequence encodes an AAV protein selected from the group consisting of: VP1, VP2, and VP3.
10. The nucleic acid molecule of claim 9, wherein the second nucleotide sequence encodes VP1, VP2, and VP3.
11. The nucleic acid molecule of claim 1, wherein the transcription product having at least one Adenoviral helper function is selected from the group consisting of: Adenovirus DNA binding protein, Adenovirus E4 protein, and Adenovirus virus associated RNA molecule.
12. The nucleic acid molecule of claim 2, wherein the nucleic acid is operably linked to at least one expression control sequence.
13. The nucleic acid molecule of claim 12, wherein the first nucleotide sequence encoding an AAV Rep protein of a first serotype is operably linked to a promoter.
14. The nucleic acid molecule of claim 13, wherein the promoter is selected from the group consisting of: AAV p5 and AAV p19 promoters.

15. The nucleic acid molecule of claim 12, wherein the second nucleotide sequence encoding an AAV Cap protein of a second serotype is operably linked to a promoter.

16. The nucleic acid molecule of claim 15, wherein the promoter is an AAV p40 promoter.

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17. The nucleic acid molecule of claim 12, wherein the third nucleotide sequence encoding a transcription product having at least one Adenoviral helper function is operably linked to a promoter.

10 18. The nucleic acid molecule of claim 1, wherein the nucleic acid molecule further comprises a selectable marker.

19. The nucleic acid molecule of claim 18, wherein the selectable marker confers antibiotic resistance to a cell.

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20. A cell comprising the nucleic acid molecule of claim 1.

21. The cell of claim 20, wherein the cell is a mammalian cell.

20 22. The cell of claim 20, further comprising a second nucleic acid comprising a polynucleotide to be expressed interposed between a first AAV inverted terminal repeat and a second AAV inverted terminal repeat.

23. The cell of claim 22, wherein the second nucleic acid is comprised within a vector.

24. The cell of claim 23, wherein the first AAV inverted terminal repeat is an AAV serotype 2 inverted terminal repeat.

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25. The cell of claim 24, wherein the second AAV inverted terminal repeat is an AAV serotype 2 inverted terminal repeat.

26. The cell of claim 22, wherein the polynucleotide encodes a protein.

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27. The cell of claim 22, wherein the polynucleotide encodes a selectable marker.

28. The cell of claim 27, wherein the selectable marker is green fluorescent protein.

15 29. A method of producing rAAV virions, the method comprising the steps of:

(a) placing the cell of claim 22 under conditions in which the nucleic acid of claim 1 is expressed, the second nucleic acid is replicated, and rAAV virions are produced; and

(b) isolating the rAAV virions produced from the cell.

20 30. The method of claim 29, wherein the cell is a mammalian cell.

31. The method of claim 29, wherein the step (a) comprises placing the cell into a culture medium.

32. The method of claim 31, wherein the step (b) of isolating the rAAV virions produced from the cell comprises separating the cell from the medium, lysing the cell to yield a cell lysate, and then isolating the rAAV virions from the cell lysate.

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33. The method of claim 29, wherein the step (b) of isolating the rAAV virions produced from the cell comprises subjecting the produced rAAV virions to an iodixanol step gradient.

34. The method of claim 33, further comprising subjecting the produced rAAV virions to ion
10 exchange chromatography.

35. The method of claim 34, wherein the produced rAAV virions contain at least one AAV serotype 1 capsid protein.

15 36. The method of claim 34, wherein the produced rAAV virions contain at least one AAV serotype 5 capsid protein.